A cultured autologous dermo-epidermal skin substitute for full-thickness skin defects: a phase I, open, prospective clinical Trial in children

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Abstract: BACKGROUND: The management of deep partial-thickness and full-thickness skin defects remains a significant challenge. Particularly with massive defects, the current standard treatment, split-thickness skin grafting, is fraught with donor-site limitations and unsatisfactory long-term outcomes. A novel, autologous, bioengineered skin substitute was developed to address this problem. METHODS: To determine whether this skin substitute could safely provide permanent defect coverage, a phase I clinical trial was performed at the University Children's Hospital Zurich. Ten pediatric patients with acute or elective deep partial- or full-thickness skin defects were included. Skin grafts of 49 cm were bioengineered using autologous keratinocytes and fibroblasts isolated from a patient's small skin biopsy specimen (4 cm), incorporated in a collagen hydrogel. RESULTS: Graft take, epithelialization, infection, adverse events, skin quality, and histology were analyzed. Median graft take at 21 days postoperatively was 78 percent (range, 0 to 100 percent). Healed skin substitutes were stable and skin quality was nearly normal. There were four cases of hematoma leading to partial graft loss. Histology at 3 months revealed a well-stratified epidermis and a dermal compartment comparable to native skin. Mean follow-up duration was 15 months. CONCLUSIONS: In the first clinical application of this novel skin substitute, safe coverage of skin defects was achieved. Safety and efficacy phase II trials comparing the novel skin substitute to split-thickness skin grafts are ongoing. CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, IV.

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A Cultured Autologous Dermo-epidermal Skin Substitute for Full-Thickness Skin Defects: A Phase I, Open, Prospective Clinical Trial in Children

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Background: The management of deep partial-thickness and full-thickness skin defects remains a significant challenge. Particularly with massive defects, the current standard treatment, split-thickness skin grafting, is fraught with donor-site limitations and unsatisfactory long-term outcomes. A novel, autologous, bioengineered skin substitute was developed to address this problem.
Methods: To determine whether this skin substitute could safely provide permanent defect coverage, a phase I clinical trial was performed at the University Children's Hospital Zurich. Ten pediatric patients with acute or elective deep partial- or full-thickness skin defects were included. Skin grafts of 49 cm^2 were bioengineered using autologous keratinocytes and fibroblasts isolated from a patient's small skin biopsy specimen (4 cm^2), incorporated in a collagen hydrogel.
Results: Graft take, epithelialization, infection, adverse events, skin quality, and histology were analyzed. Median graft take at 21 days postoperatively was 78 percent (range, 0 to 100 percent). Healed skin substitutes were stable and skin quality was nearly normal. There were four cases of hematoma leading to partial graft loss. Histology at 3 months revealed a well-stratified epidermis and a dermal compartment comparable to native skin. Mean follow-up duration was 15 months.
Conclusions: In the first clinical application of this novel skin substitute, safe coverage of skin defects was achieved. Safety and efficacy phase II trials comparing the novel skin substitute to split-thickness skin grafts are ongoing. (*Plast. Reconstr. Surg.* 144: 188, 2019.)
CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, IV.

The management of deep partial-thickness and full-thickness skin defects remains challenging, with a broad spectrum of clinical problems ranging from rare, massive, deep burns in healthy patients to common, small ulcers in elderly people with multiple morbidities. The common denominator is the need for permanent skin coverage that provides the patient with a long-term result that is stable and functionally and aesthetically optimal.

Small defects can be covered with full-thickness skin grafts harvested from the patient with usually excellent results. However, there is a marked donor-site shortage. Thus, for larger defects, most notably

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burns, split-thickness skin grafts remain the standard treatment, often applied with expansion techniques, such as meshing or Meek micrografting.[1,2] Here, the results are less favorable, with a risk of functionally debilitating and disfiguring scars, particularly in children.[3] Furthermore, split-thickness skin grafts increase the patient's wound healing burden, and, in massive burns, donor-site limitations cause delays in definitive coverage that may lead to sepsis, multiple organ dysfunction, and death.[4]

Clearly, culturing epidermis from the patient's own cells, as envisioned by Rheinwald and Green 40 years ago,[5,6] was revolutionary and has fostered intense research during recent decades. Unfortunately, cultured epithelial autografts remain fraught with considerable problems when used for the coverage of deep skin defects, including graft fragility, poor take, instability of healed grafts, and unsatisfactory long-term functional and aesthetic results.[2,7–9] The clinical introduction of nowadays widely used dermal regeneration templates [e.g., Integra Dermal Regeneration Template (Integra LifeSciences Corp., Plainsboro, N.J.), Matriderm (MedSkin Solutions Dr. Suwelack AG, Billerbeck, Germany)] has pushed the frontiers further, carrying the potential for improved functional and aesthetic results, yet such templates still require coverage with an overlying autologous split-thickness skin graft.[10]

In the early 1990s, the first cultured autologous dermo-epidermal skin substitute, developed by Hansbrough et al., was successfully applied clinically for "compassionate use" in severe burns,[11] and has more recently been investigated in a clinical trial setting.[12] However, more than 25 years since its introduction, this skin analogue is still not commercially available. Another bioengineered dermo-epidermal skin substitute, developed at LOEX (Quebec City, Quebec, Canada), was introduced in preclinical studies[13] and as biological dressing.[14]

Here, we report on a novel, bioengineered, hydrogel-based, autologous, dermo-epidermal skin substitute used in a first-in-human clinical trial in children. These grafts are based on plastically compressed collagen type I hydrogels with incorporated keratinocytes and fibroblasts that proliferate and differentiate. It features an epidermis that correctly stratifies on grafting, seems to develop a functional basement membrane and dermo-epidermal junction, and demonstrates a nearly normal, functional dermis. The results of the preclinical studies on immunoincompetent rats and a porcine large-animal model strongly support the envisioned application in humans.[15–19]

Collagen hydrogels have been used as experimental tissue scaffolds[20] and in the commercially available allogeneic skin substitute Apligraf (Organogenesis, Canton, Mass.). However, there are no reports of a hydrogel-based skin substitute composed of purely autologous cells that safely and permanently replaces skin on human patients.

PATIENTS AND METHODS

Study Design and Participants

Ten patients (defined using power analysis) presenting to the Division of Plastic and Reconstructive Surgery at the University Children's Hospital Zurich for management of deep partial- or full-thickness skin defects, or reconstruction of skin lesions, were recruited for this phase I clinical trial. No rules to stop data collection in advance were defined. Inclusion and exclusion criteria and the study flow chart are outlined in Figure 1. Outliers were included in the analysis.

This clinical trial conforms to the Declaration of Helsinki and the guidelines for Good Clinical Practice and follows the European Medicines Agency guidelines for Advanced Therapy Medicinal Products. The trial was approved by the local ethical committee Zurich (KEK-ZH-Nr.2012-0573) and by Swissmedic (2013TpP1004). All patients/legal representatives received detailed information about the study. Biopsies, operations, and all associated procedures were performed after informed consent was obtained.

The main objective was safety evaluation of the skin substitute. The experimental design was a phase I, two-armed, open, prospective study to evaluate the safety of autologous tissue-engineered dermo-epidermal skin substitutes for the treatment of large deep partial- and full-thickness skin defects in children and adolescents. The experimental arm was transplantation of 49 cm^2 of the novel graft. The primary endpoints were graft take at 21 days postoperatively and infection rate, and the secondary endpoint was assessment and reporting of adverse events.

Biopsy

A split-thickness skin biopsy specimen measuring 4 cm^2 was harvested from the retroauricular scalp using an electric dermatome, with either local or general anesthesia, depending on the child's age and the need for concomitant surgery.

Skin Manufacturing

Manufacturing, in accordance with guidelines for Good Manufacturing Practice (Swissmedic no.

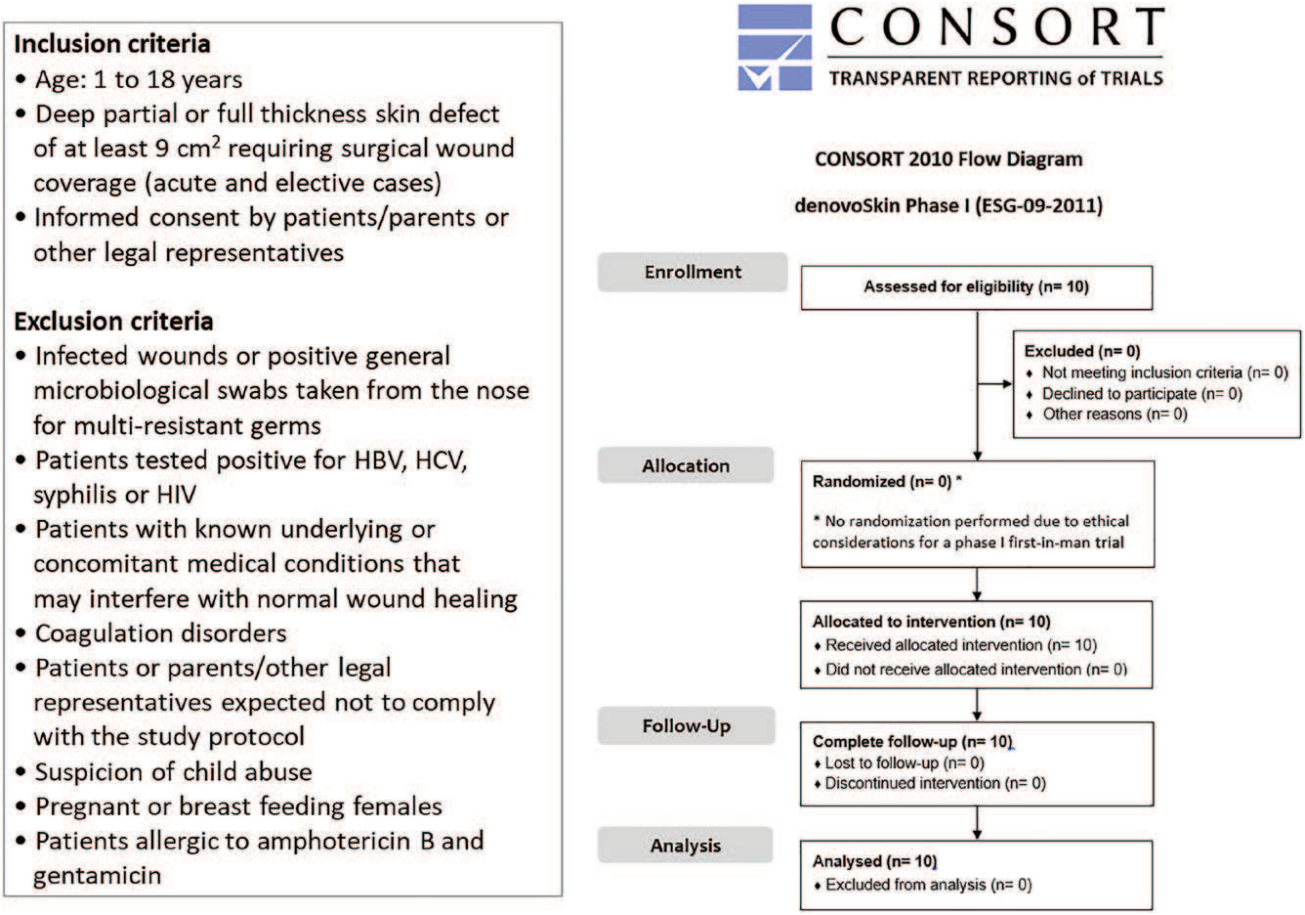

Fig. 1. Inclusion/exclusion criteria and study cohort flow chart. (*Left*) Inclusion and exclusion criteria. (*Right*) Study cohort flow chart. *HBV*, hepatitis B virus; *HCV*, hepatitis C virus; *HIV*, human immunodeficiency virus.

510793; Wyss Zurich, Zurich, Switzerland) was performed as described previously.[16,18,21] Briefly, dermis and epidermis were separated enzymatically (Fig. 2, *above*, *left*), followed by fibroblast and keratinocyte isolation and expansion (Fig. 2, *second row*, *left*, and *second row*, *right*).[22,23] Keratinocytes were cultured in serum-free media (CELLnTEC Advanced Cell Systems AG, Bern, Switzerland), and fibroblasts were cultured in Dulbecco's Modified Eagle Medium (Sigma-Aldrich, Buchs, Switzerland) containing 10% fetal bovine serum (Life Technologies, Zug, Switzerland). For graft production, fibroblasts were incorporated into an acid-soluble bovine collagen type I hydrogel (Symatese, Chaponost, France) as described previously.[18,21,24] After plastic compression of the hydrogel and cultivation for 5 to 6 days, keratinocytes were seeded at a density of approximately $0.15 \times 10^6/cm^2$ onto the surface of the graft.[18,22–24] After additional cultivation,[18,22,23] grafts (Fig. 2, *above*, *right*) were shipped to the study site in a cell culture medium–filled tissue culture flask in a temperature-regulated transport box at 35 ± 5°C, to be grafted within 24 hours. Graft size was 45 ± 4 cm², with a thickness of 1 ± 0.5 mm.

Routine testing for sterility, mycoplasma, and endotoxins according to the European Pharmacopoeia were undertaken throughout the manufacturing process. Cell identity and purity were confirmed using flow cytometry (Fig. 2, *fourth row*, *left*, and *fourth row*, *right*). Quality control tests included size and thickness measurements (Fig. 2, *above*, *right*), assessment of cell number (DNA count), cell viability/distribution (fluorescein diacetate staining) (Fig. 2, *third row*, *left*, and *third row*, *right*), and histology (hematoxylin/eosin staining) (Fig. 2, *below*).

Grafting Procedure

Grafting was performed by the principal investigator (M.M.) and coordinating investigator (C.S.) under general anesthesia with sterile conditions. In acute cases, contaminated and devitalized tissues were débrided (Fig. 3, *above*, *left*), and wound bed conditioning with temporary allograft coverage was performed to ensure

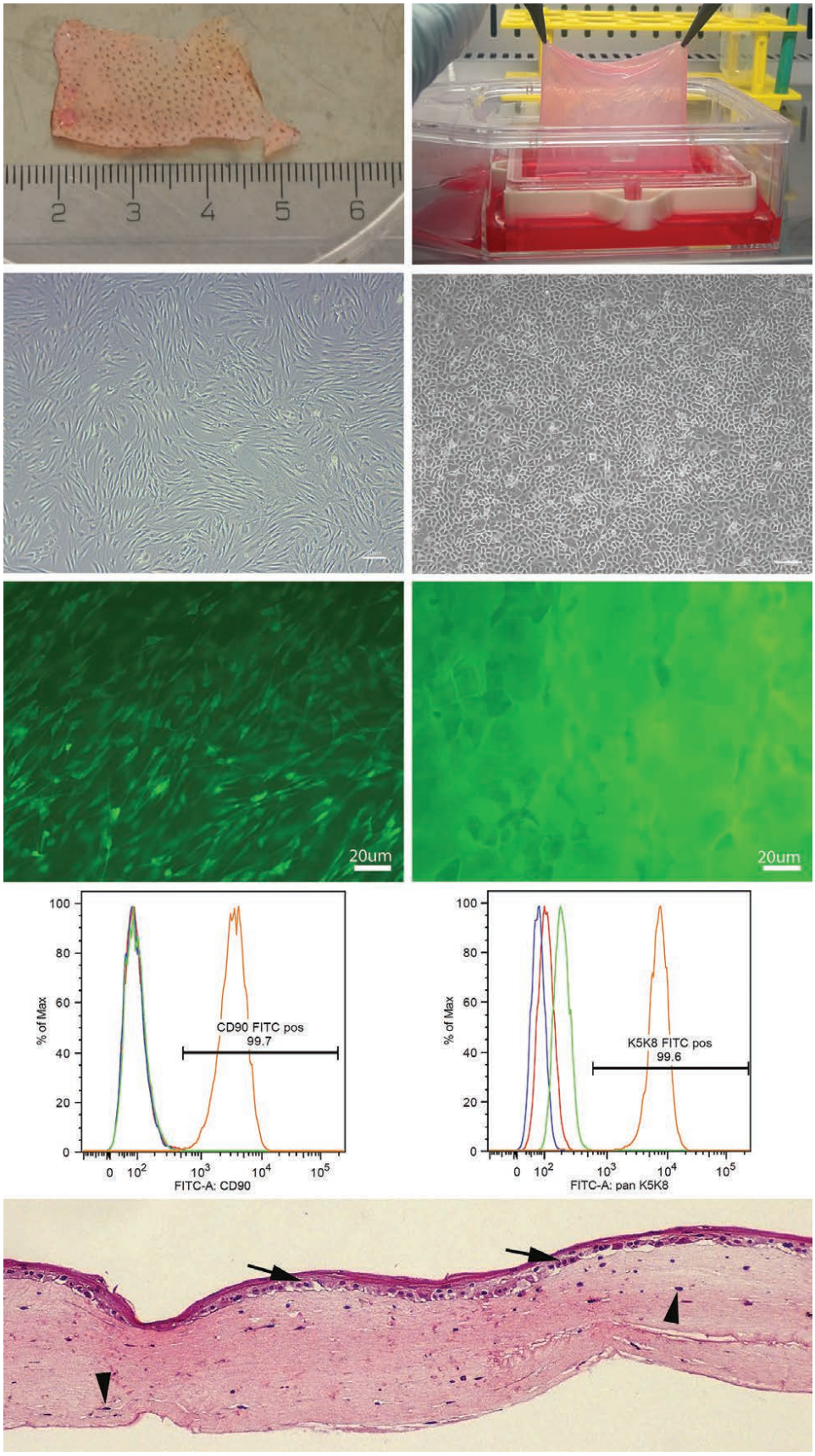

Fig. 2. (*Continued*).

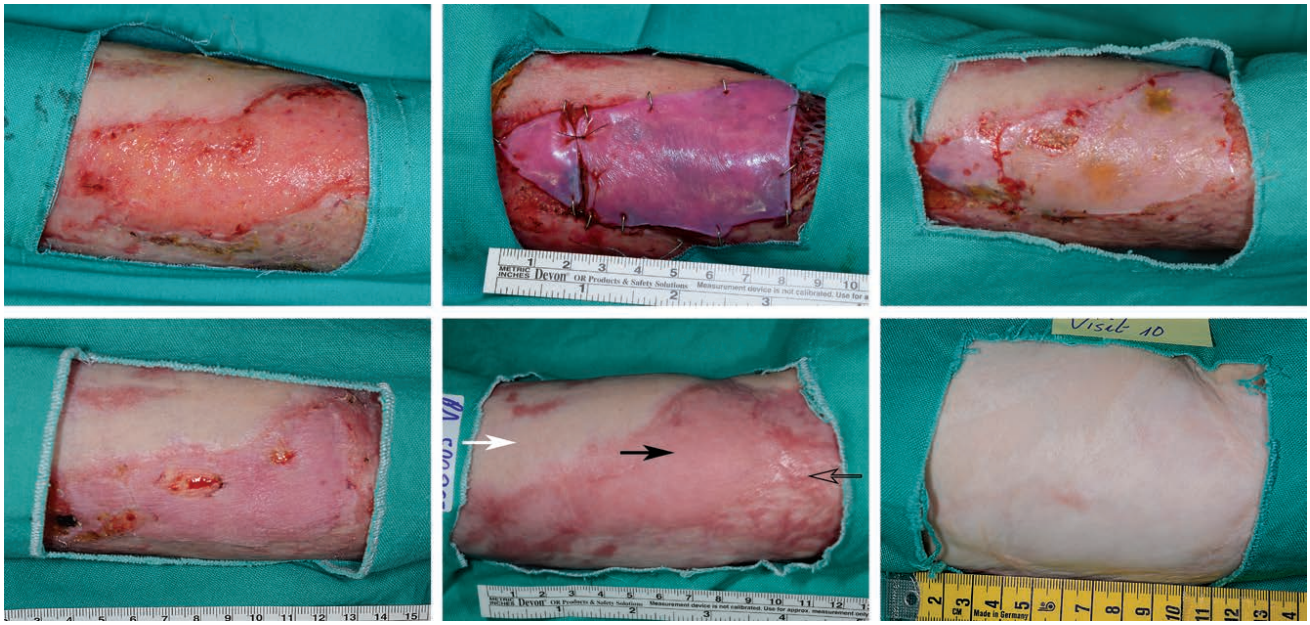

Fig. 3. Clinical course of the burn patient. (*Above*, *left*) Patient 3 intraoperatively after wound bed preparation. (*Above*, *center*) After application of the bioengineered skin. (*Above*, *right*) Postoperative appearance at day 8. (*Below*, *left*) After 3 weeks. (*Below*, *center*) After 3 months. Please note the grafted bioengineered skin (*black arrow*) in comparison to the gold standard, meshed split-thickness skin graft (*open arrow*), and unaffected, healthy skin (*white arrow*). (*Below*, *right*) After 2 years. The transplanted skin graft is almost indistinguishable from healthy skin and is larger when compared to *below*, *center, white arrow*.

a well-vascularized and sterile recipient site. In reconstructive cases, the area of pathology was excised, creating either a partial- or a full-thickness defect.

Fig. 2. (*Continued*). Bioengineering process of the autologous, dermo-epidermal skin substitute. (*Above*, *left*) Split-thickness skin biopsy specimen (macroscopic view). (*Above*, *right*) The 7 × 7-cm bioengineered skin before grafting, grown in a specially designed culture flask. (*Second row*, *left*) Phase-contrast micrograph of dermal fibroblasts expanded on cell culture plastic. (*Second row*, *right*) Fluorescein diacetate staining of living dermal fibroblasts in the hydrogel. (*Third row*, *left*) Fluorescence-activated cell sorting analysis performed on human dermal fibroblasts stained by the fibroblast-specific antibody to CD90 (*red*, unstained control; *green*, isotype control; *yellow*, CD90-specific antibody). *FITC*, fluorescein isothiocyanate. (*Third row*, *right*) Phase-contrast micrograph of human epidermal keratinocytes expanded on cell culture plastic. (*Fourth row*, *left*) Fluorescein diacetate staining of a confluent monolayer of living keratinocytes on the upper surface of the hydrogel. (*Fourth row*, *right*) Fluorescence-activated cell sorting analysis performed on human epidermal keratinocytes stained by the specific keratin markers K5/K8 (*red*, unstained control; *green*, isotype control; *yellow*, K5/K8-specific antibody). (*Below*) Hematoxylin and eosin staining of a section of the bioengineered skin, before grafting. *Arrowheads* indicate fibroblasts in the dermal compartment. *Arrows* indicate keratinocyte layers on top of the bioengineered skin. Please note the presence of the faint stratum corneum.

Hemostasis was achieved with bipolar cautery and epinephrine-soaked gauzes, and the wound bed was sprayed with fibrin sealant (ARTISS; Baxter Healthcare, Deerfield, Ill.). Grafts were cut to fit the defect (mean, 41 cm^2; range, 32 to 49 cm^2), secured in place using staples (Fig. 3, *above*, *center*), and dressed with Mepilex (Mölnlycke Health Care, Gothenberg, Sweden) and an overlying occlusive dressing or a negative-pressure device (KCI Vacuum Assisted Closure; Kinetic Concepts, Inc., San Antonio, Texas). Remaining defects were grafted in accordance with the current standard of care.

The first dressing change was carried out between postoperative days 9 and 11. Thereafter, in uncomplicated cases, patients were discharged with outpatient follow-up and photographic documentation at 2, 3, and 4 weeks; 2 and 3 months; 1 year; and then annually for 5 years thereafter.

Outcome Measures

The primary outcome was "safety" (local infection and graft take/epithelialization), and the secondary outcome was "adverse events." Percentage graft take, assessed at the time of the first dressing change, and percentage epithelialization, assessed at 3 weeks, were determined as follows: standardized photographic documentation, delineation of epithelialized and nonepithelialized areas on the

photographs by an experienced observer (M.M. or C.S.), and percentage calculation using computerized planimetry as proposed by Bloemen et al.[25] and Boyce et al.[26]

Scar quality was assessed with the Patient and Observer Scar Assessment Scale at 1 year postoperatively and annually thereafter (for 5 years). Briefly, the Patient and Observer Scar Assessment Scale is a validated scar assessment tool that consists of a patient scale and an observer scale, whereby scar characteristics are rated from 1 to 10 (where 1 = normal skin and 10 = worst imaginable scar or sensation), and the total scores for each scale are calculated.[27]

All adverse events were handled according to the Good Clinical Practice guidelines from the International Conference on Harmonisation and the Swiss Federal Council's Regulation on Clinical Trials with Medicinal Products. Histologic analyses of grafted areas were carried out with an optional biopsy at 3 months postoperatively.

Histology

Punch biopsy specimens (3-mm diameter) were processed for histology and immunofluorescence as described previously.[24] Antibodies included anti-human K1 (Novus Biologicals, Littleton, Colo.), anti-human Lam332 (Santa Cruz, Nunningen, Switzerland), anti-human Tropoelastin (Elastin Products Co., Owensville, Mo.), anti-human CD31 (Dako, Switzerland), and anti-human Keratin19 (Dako, Switzerland).

RESULTS

Patient Demographics

Between July of 2014 and March of 2016, six male and four female patients, aged 8 to 18 years, underwent grafting (Table 1). There was one patient with acute burns, whereas nine patients underwent reconstructive surgery, primarily for burn scars ($n = 7$), at a mean of 9 ± 4 years (range, 7 to 14 years) after injury. The mean follow-up duration was 15 ± 7 months (range, 2 to 25 months).

Manufacturing

Cell isolation and expansion from split-thickness skin biopsy specimens (Fig. 2) was successful in all 10 biopsy specimens. Grafts were produced in a mean ± SD of 32 ± 4 days (range, 26 to 38 days). All 10 grafts fulfilled the in-process controls and release criteria. Grafts maintained their size and allowed handling with forceps (Fig. 2).

Pretransplant Histology

Before transplantation, grafts showed an even distribution of viable fibroblasts in the dermal compartment (Fig. 2, *third row, left*) and a confluent layer of viable keratinocytes on the surface (Fig. 2, *third row, right*). Hematoxylin and eosin staining confirmed the presence of cells in the dermal and epidermal compartments (Fig. 2, *below*).

Outcomes

Main results are shown in Table 1. Briefly, mean graft take at days 9 through 11 was 67 ± 32 percent (range, 0 to 100 percent; median, 65 percent). Patient 9 manipulated the dressing postoperatively and suffered total graft loss. Subanalysis excluding this patient yielded a mean take of 74 ± 23 percent (range, 50 to 100 percent; median, 75 percent). Mean epithelialization at day 21 was 63.5 ± 35 percent (range, 0 to 98 percent; median, 78 percent). When excluding the noncompliant patient, mean epithelialization at day 21 was 70.6 ± 30.6 percent (range, 5 to 98 percent; median, 80 percent). There were no cases of infection. Donor-site healing was uneventful in all cases.

Regarding adverse events, there were four cases of hematoma, three of which healed spontaneously (patients 1, 4, and 7), whereas one required repeated split-thickness skin grafting (patient 10). The patient with total graft loss also underwent regrafting with split-thickness skin graft.

Figure 3 illustrates the clinical course of the acute burn patient. After wound bed preparation (Fig. 3, *above, left*), the cultured graft was transplanted next to meshed split-thickness skin graft and unwounded skin (left arm) Fig. 3, *above, center*). At 8 and 21 days after transplantation, the take was 100 percent (Fig. 3, *above, right* and Fig. 3, *below, left*). After 3 months, the bioengineered skin (Fig. 3, *below, center, black arrow*) was smoother and more pliable than the neighboring meshed split-thickness skin graft (Fig. 3, *below, center, open arrow*) and, except for the redness, was very similar to the adjacent normal skin (Fig. 3, *below, center, white arrow*). After 2 years, the cultured skin (Fig. 3, *below, right*) was almost indistinguishable from normal skin (Fig. 3, *below, center, white arrow*) and had notably grown compared with the size at 3 months (Fig. 3, *below, center*). Patient and Observer Scar Assessment Scale scores 1 year postoperatively for the first eight patients and 2 years postoperatively for the first three patients were obtained, as presented in Table 2.

Table 1. Patient Summary

Patient	Age (yr)	Sex	Diagnosis	Time after Injury (yr)	Operative Site	Operative Area (cm^2)	Operative Details	% Graft Take (day 9–11)	% Epithelialization (day 21)	Infection	Complications	Reoperation	Follow-Up Duration (mo)
1	15	Male	Burn scar*	13.70	Left arm	42	PT tangential excision of scar; dermal overgrafting with BESS	50	76	No	Hematoma	No	25.17
2	17	Female	Burn scar*	8.27	Left thigh	45	PT tangential excision of scar; dermal overgrafting with BESS	100	86	No		No	23.07
3	8	Male	Acute burn	0.13	Left arm	46	Staged procedures: FT tangential excision, allograft; removal of allograft, tangential excision of surface granulation tissue, BESS application	100	90	No		No	25.37
4	18	Male	Giant CMN	NA	Left back	36	FT intralesional excision of nevus; BESS application	55	50	No	Hematoma	No	12.47
5	14	Female	Burn scar	6.84	Left chest	49	PT tangential excision of scar; dermal overgrafting with BESS	100	98	No		No	11.37
6	15	Female	Scar contracture, asymmetric breast development	NA	Left chest	44	FT scar excision, release of tethered left breast; BESS application	50	100	No		No	13.27
7	11	Male	Burn scar	11.27	Left forearm	42	PT tangential excision of scar; dermal overgrafting with BESS	50	50	No	Hematoma	No	12.53
8	14	Female	Burn scar	13.21	Right chest	Approximately 36	PT tangential excision of scar; dermal overgrafting with BESS	90	80	No		No	12.87
9	11	Male	Burn scar	9.80	Left chest	Approximately 32	PT tangential excision of scar; dermal overgrafting with BESS	0	0	No	Manipulation of dressing by patient, 100% graft loss	Yes, STSG	12.20
10	11	Male	Burn scar*	10.43	Left chest	39	PT tangential excision of scar; dermal overgrafting with BESS; VAC application	75	5	No	Late-onset hematoma, 95% graft loss	Yes, STSG	2.97

CMN, congenital melanocytic nevus; FT, full-thickness; PT, partial-thickness; POD, postoperative day; BESS, bioengineered skin substitute; STSG, split-thickness skin graft.
*Widely meshed pattern.

Histology

Three months postoperatively, there was a multilayered, well-stratified epidermis and a dermal compartment comparable to native skin (Fig. 4, *above*, *left*, and *above*, *center*). Immunofluorescence staining for the basement membrane component Laminin332 revealed the deposition of a continuous basement membrane (Fig. 4, *above*, *right*). Keratin 1 expression was found in all suprabasal layers of the epidermis (Fig. 4, *above*, *right*). In the epidermis, a subpopulation of basal cells were keratin 19–positive (Fig. 4, *below*, *left*). Blood vessels were found throughout the dermis and in close proximity to the dermal-epidermal junction, as visualized by staining for CD31 (Fig. 4, *below*, *center*). Tropoelastin could be detected throughout the dermis up to the dermoepidermal junction (Fig. 4, *below*, *right*).

DISCUSSION

This is a first-in-human clinical trial to evaluate whether a bioengineered, hydrogel-based, autologous dermo-epidermal skin substitute can be safely used for permanent coverage of skin defects in children and adolescents. Favorably, in the present setting of nine reconstructive (elective) cases and one acute burn case, there were no safety-related issues recorded. Of note, because patient safety was the primary outcome, we deliberately chose to treat easily accessible areas, to avoid location-associated problems (e.g., neck, axilla, thigh). In addition, in the global picture, grafting of this novel study product yielded promising functional and aesthetic long-term outcomes (Fig. 3), with nearly normal skin architecture histologically (Fig. 4).

This clinical trial focused exclusively on children and adolescents, acknowledging that they demonstrate quite high rates of burn injuries and other conditions associated with extensive skin loss. In addition, children are more susceptible to hypertrophic scarring,[3] and they are facing devastating consequences of growth in the presence of unyielding scars. Theoretically, they could benefit immensely—and notably, more than adults—from a skin graft that heals with only mild scarring and that grows proportionally with the patient.

From a small (4-cm^2) skin biopsy specimen, a protocol-specified 49-cm^2 piece of skin was generated in approximately 1 month. Of note, this manufacturing process would also allow a 70-fold expansion (i.e., production of 280 cm^2) within the same production time, thereby offering a potentially life-saving intervention (e.g., in a small child

Table 2. Patient and Observer Scar Assessment Scale Scores at 1 and 2 Years after Grafting with the Bioengineered Skin Analogue

	Observer Scale								Patient Scale							
Patient	Total Score	Vascularity	Pigmentation	Thickness	Relief	Pliability	Surface Area	Overall Opinion	Total Score	Pain	Itching	Color	Pliability	Thickness	Relief/ Irregularity	Overall Opinion
							1-Yr Follow-Up									
1	10.00	2.00	2.00	2.00	1.00	1.00	2.00	2.00	18.00	1.00	1.00	6.00	5.00	4.00	1.00	3.00
2	12.00	3.00	5.00	1.00	1.00	1.00	1.00	1.00	12.00	1.00	1.00	7.00	1.00	1.00	1.00	4.00
3	16.00	2.00	4.00	5.00	2.00	2.00	1.00		14.00	1.00	5.00	2.00	2.00	2.00	2.00	2.00
4	31.00	7.00	5.00	2.00	5.00	5.00	7.00	6.00	21.00	1.00	1.00	5.00	5.00	5.00	4.00	6.00
5	26.00	5.00	4.00	5.00	3.00	3.00	6.00	5.00	32.00	5.00	1.00	10.00	6.00	5.00	5.00	8.00
6	30.00	2.00	2.00	4.00	6.00	8.00	8.00	8.00	17.00	3.00	1.00	2.00	6.00	2.00	3.00	5.00
7	13.00	3.00	3.00	2.00	2.00	2.00	1.00	3.00	20.00	1.00	1.00	8.00	4.00	3.00	3.00	8.00
8	17.00	4.00	1.00	2.00	4.00	3.00	3.00	4.00	16.00	1.00	1.00	5.00	4.00	2.00	3.00	5.00
Mean	19.38	3.50	3.25	2.88	3.00	3.13	3.63		18.75	1.75	1.50	5.63	4.13	3.00	2.75	
SD	8.38	1.77	1.19	1.55	1.85	2.36	2.92		6.11	1.49	1.41	2.77	1.81	1.51	1.39	
							2-Yr Follow-Up									
1	8	1.00	3.00	1.00	1.00	1.00	1.00	1.00	19.00	1.00	1.00	6.00	3.00	7.00	1.00	3.00
2	16.00	3.00	6.00	1.00	1.00	1.00	1.00	3.00	11.00	1.00	1.00	6.00	1.00	1.00	1.00	4.00
3	6.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	9.00	1.00	1.00	3.00	1.00	2.00	1.00	1.00

*Scar quality evaluated with Patient and Observer Scar Assessment Scale item scores (i.e., vascularity, pigmentation, thickness, relief, pliability, and surface area), rated from 1 to 10 (where 1 = normal skin and 10 = worst imaginable scar), total score, and overall opinion at 12 and 24 mo after grafting with the bioengineered skin analogue. Overall mean and standard deviation are indicated.

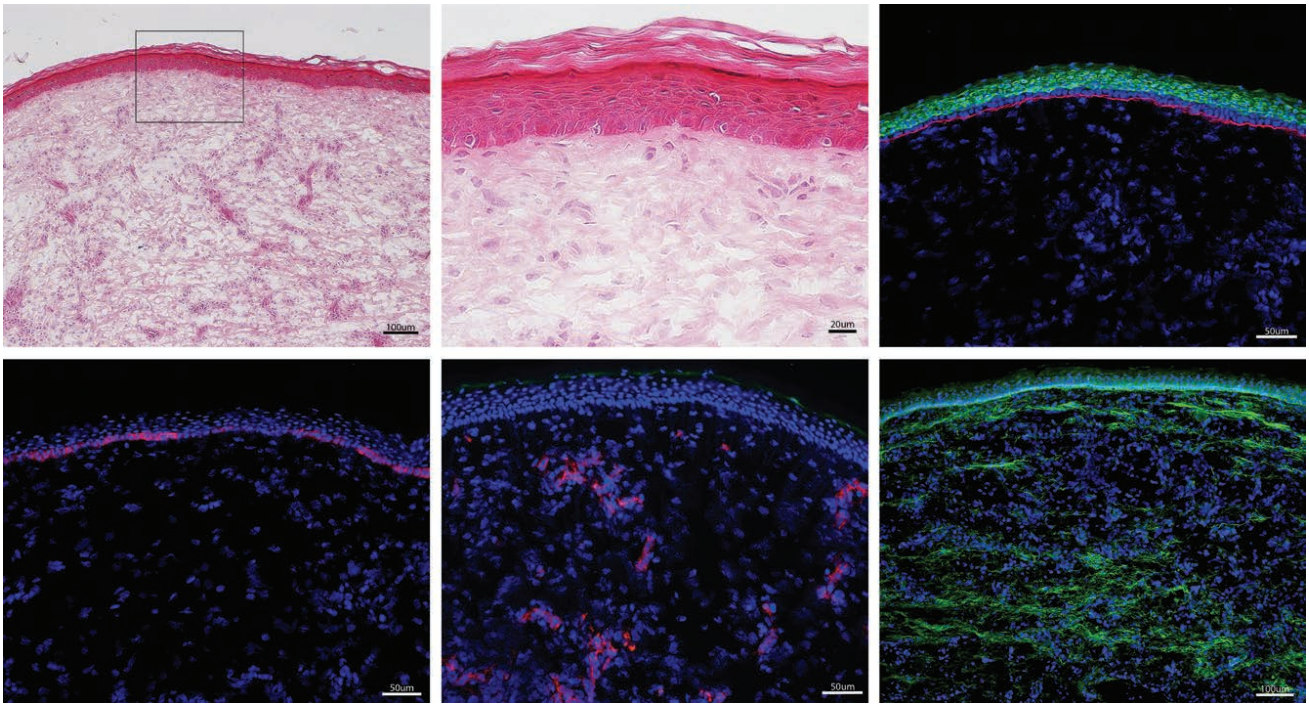

Fig. 4. Analyses of punch biopsy specimens reveal functional skin, 3 months after grafting. (*Above*, *left*) Hematoxylin and eosin staining of a 3-mm punch biopsy specimen of the grafted bioengineered skin. Note the functional dermis and the stratified epidermis covered by a stratum corneum. (*Above*, *center*) Magnification of the *inset* (*gray frame*) in *above*, *left*. (*Above*, *right*) Staining of the continuous basement membrane by an antibody to laminin-332 (*red line*). A K1 antibody (*green*) stains suprabasal keratinocyte layers, whereas the basal layer is K1-negative. Nuclei stained by Hoechst dye. (*Below*, *left*) As expected, and described by us previously, K19 expression is restricted to the basal layer. (*Below*, *center*) A well-organized dermal capillary plexus is detected by an antibody to human-specific CD31 (*red*). (*Below*, *right*) Tropoelastin (*green*) is reexpressed in the dermis, 3 months after grafting. Nuclei are stained by Hoechst dye.

with massive burns). This production time, even though seemingly long, is similar to the 4 to 5 weeks required for the skin analogue developed by Boyce et al.[12] and significantly faster than the 8 weeks needed for the skin analogue produced by Auger et al.[13] Nevertheless, it is our explicit goal to reduce production time so that more acute cases become eligible for this innovative approach.

The preoperative graft histology reveals a one- to three-layer epidermis, associated with a collagen- and fibroblast-rich dermis (Fig. 2, *below*). This translates clinically to grafts that are pliable and robust enough to be handled surgically. Furthermore, in accordance with our preclinical studies,[15] grafts become readily vascularized (Fig. 4, *below*, *center*). Postoperatively, grafts have matured quickly and without scar hypertrophy (Fig. 3). Long-term follow-up demonstrates remarkable stability of the grafted skin, with no findings of blistering or wound breakdown. This may be attributed to an evolving (i.e., possibly not yet complete and fully mature) but already functionally competent dermoepidermal junction (Fig. 4, *above*, *right*)[8] and a population of self-renewing cells in both dermis and epidermis.[21]

The skin quality of grafted areas compares favorably with conventional split-thickness skin grafting, as demonstrated visually (Fig. 3) and supported by Patient and Observer Scar Assessment Scale scores 1 year postoperatively (Table 2). The parameters relevant for hypertrophic scarring (i.e., pliability, thickness, and relief) in the present study were scored better than in a large study of 474 burn patients (284 children) 1 year after split-thickness skin graft transplantation.[28] Importantly, subanalysis comparing Patient and Observer Scar Assessment Scale scores between adult and pediatric patients did not reveal significant differences.[28] Reassuringly, Patient and Observer Scar Assessment Scale scores for pain (mean, 1.75; SD, 1.49) and itching (mean, 1.50; SD, 1.41) were also lower than in the quoted study.[28] Not astonishingly, the least favorable Patient and Observer Scar Assessment Scale scores were seen for the patient parameter "skin color." The reason is hypopigmentation that unequivocally occurs because of the paucity

of melanocytes whenever cultured skin is applied clinically.[12,16]

Our cultured skin analogue contains the fundamental elements of skin, a nearly natural epidermis and dermis, and a dermo-epidermal junction that, from a clinical perspective, appears functionally competent. However, like the very few comparable versions of bioengineered and clinically applied dermo-epidermal skin analogues, it also lacks crucial components, including melanocytes, skin appendages, and a neurovascular supply. We carried out preclinical studies to create a more complete skin substitute and demonstrated the feasibility of producing and transplanting pigmented[16] and prevascularized[17] skin grafts. Correspondingly, first-in-human studies are planned.

In the current study, the mean graft take rate appears somewhat lower than rates typically seen with conventional split-thickness skin grafts.[12] The instances where incomplete take and graft loss were higher than expected were mainly attributable to the presence of hematomas,[29] rather than intrinsic graft problems. Clearly, these hematoma complications were also the key factors for the relatively low epithelialization rates seen at 21 days. We encountered this vexing and rather unexpected problem despite choosing flat and relatively immobile areas for grafting; ensuring meticulous hemostasis; and applying fibrin glue, the best possible graft fixation, and optimal dressings. We hypothesize that hematoma formation results from less efficient clotting cascade triggers in the bioengineered skin compared with split-thickness skin graft. Patient medications and laboratory coagulation parameters were formally reviewed with a hematologist, but no differences between the groups (with or without hematomas) were observed. Graft take rates were, however, similar to rates seen in clinical studies of Boyce et al.,[26] and also comparable to rates seen when dermal regeneration matrices were applied to burn wounds, together with an overlying thin split-thickness skin graft.[30] Along the usual learning curve that characterizes these types of innovative approaches, refinements will be made to both technique and perioperative management to improve take rates in our follow-up studies. Currently, three international phase II trials (NCT03394612, NCT03229564, and NCT03227146) are ongoing to evaluate the study product in a randomized intrapatient controlled setting.

The main limitation of this study lies in the ethical and regulatory requirements for a first-in-human phase I study. The outcome variables were given and limited. With safety as the primary outcome, the formal inclusion of a control was not feasible. However, our currently running phase II clinical trials are designed as intrapatient randomized controlled studies (see above).

Based on the same ethical considerations for a phase I study, the number of patients was small and the area allowed to graft was formally limited to 49 cm^2. Phase II trials will have to show what occurs when significantly larger areas are grafted with the study product.

Furthermore, nine of the 10 patients treated were elective cases, whereas only one was a burn patient. Even though this burn patient demonstrated a most favorable course and final outcome, the efficacy of the cultured graft for the treatment of acute burns must be demonstrated in a larger number of acute patients.

Lastly, this phase I clinical trial is only a preliminary, initial analysis of a few fundamental points—in this case, primarily safety and adverse events. The present study design does not allow production of much more evidence than proof of principle: clinical application of the study product is feasible and safe. All other intriguing issues such as details of clinical efficacy (in particular, large-scale transplantation and application in demanding areas), comparison with other methods, range of indications, potential improvements, cost-effectiveness, and more, must be and will be addressed in subsequent trials.

CONCLUSIONS

This phase I clinical trial demonstrates that a laboratory-engineered, autologous, hydrogel-based, dermo-epidermal skin substitute can be used safely to permanently cover skin defects in children and adolescents. There is preliminary evidence that the study product may compare favorably with split-thickness skin grafting in terms of both functional and aesthetic long-term results. Moreover, this study paved the way for phase II clinical trials. Finally, the promising results of the present study highlight the potential of this novel bioengineered skin substitute to become both a life-saving therapy for massive skin defects and a viable new approach to treat myriads of patients of all ages requiring surgical coverage of a broad range of skin defects.

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